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Cephalosporium elm wilt in Massachusetts

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CEPHALOSPORIUM ELM WILT IN MASSACHUSETTS

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INTRODUCTION

The widespread occurrence, in Massachusetts, of a wilt disease of American elm (Ulmus americana L.) associated with a fungus belonging to the genus Cephalosporium, Corda, became apparent to members of the staff in the Shade Tree Disease Laboratory at Massachusetts State College during recent surveys of shade tree pests. Because of the limited extant knowledge concerning this disease it was considered advisable to make further investigations of Cephalosporium wilt in elms. Therefore, the writer has undertaken studies concerning the course of the disease in different species and varieties of elm, the relation of the fungus pathogen to the host, (including the possible means of fungus entrance, fungus development in the host tissues, and the progress of parasitic growth), and data concerning the morphology, physiology, and life-history of the organism. The present report describes the results of these investigations in comparison with previously published reports by various investigators.

Ulmus americana is the most widely planted shade tree in New England and is generally considered to be one of our most beautiful trees. It has been widely used in street planting since early colonial days and the majority of our old Massachusetts towns have grown up around elm-shaded commons. The elm is as much a part of the tradition of Massachusetts, and New England, as are the familiar white church spires which

dot the country-side. Not only are the elms a cherished heritage, but also they possess a unique beauty, which, in the eyes of many, transcends that of any other tree. Moreover, the charm of Massachusetts' elm-planted villages is one of its greatest commercial assets. This has not long been recognized, but it is becoming increasingly appreciated as tourist, recreation and leisure time activities increase. It is apparent that this disease of the elm deserves much careful study.

REVIEW OF LITERATURE

The genus Cephalosporium, does not figure prominently in phytopathological literature, but several species have been reported as occurring saprophytically. Adams and Manns (2)[1] (1922) reported C. sacchari Adams & Manns, as following corn ear worm in the kernel rot of corn. Young (17) (1926) describes C. acremonium, Corda in connection with callosites on garden tuck. Abbot (1) (1929) attributes a leaf spot of coffee to Cephalosporium sp. Corda, but considers it of minor importance. In England, C. malorum K. and Beaum, was described by Kidd and Beaumont (9) (1924) as the cause of rots on apples in cold storage. Another species, C. carpoginum Ruehle, was found on apple fruit in the United States by Ruehle (15) (1931). Morrow (12) (1932) claims that C. curtipes Sacc. is very common in forest soils, and Paine (14) (1929) finds that members of the genus act as cellulose destroyers.

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1. The numbers in parentheses, in this paper refer to corresponding titles listed in the bibliography.

Muller (13) in 1933 reported C. lecanii Zimm. on scale insects of citrus in Brazil, and suggested its cultivation as an insect control measure.

So far as the writer can learn, the only instance reported of any species of the genus Cephalosporium occurring as an important plant parasite is in association with the wilt or die-back of elm. This disease was discovered rather recently and the literature concerning it is very limited. In 1931 May (11) described a new elm disease which he attributed to infection by the fungus Cephalosporium sp. Of 300 specimens received by him during the previous year ten percent were found to be infected with this fungus. He describes the symptoms as very similar to those of the disease caused by species of Graphium and Verticillium. The cultures which he obtained were white, cottony colonies of aerial mycelium, later becoming light brown; spores were hyaline, generally elliptic, variable in shape, one or two oil drops, and an average size of 1.9 x 4.5 microns. His specimens had been collected from Iowa, Missouri, New York, Ohio and Washington, D. C.

Liming (10) (1933) found that trees affected with Cephalosporium sp. show an increase in the diseased condition in the second year. He also stated that out of 1407 trees suspected of the Dutch Elm Disease 336 showed a fungus referred to Cephalosporium.

In 1934, Goss and Frink (7) published an account of their studies of the disease. They found the disease to be common in the city of Lincoln, Nebraska. They inoculated both U. americana and U. pumila L. but were not successful with the latter. They found, in the former, that the organism progressed more rapidly upward than toward the root. Greater success was obtained in their experiments when inoculating into roots and trunks, than when inoculating soil or leaves.

In a note published in 1935, Creager (5) reports the occurrence of pycnidia, both in nature and in laboratory cultures. He also reports that, according to his experiments, the most common method of entrance is through wounds in the leaves.

Beattie (4) reports that while the disease seems to be very prevalent in the United States, it is unknown in England.

The writer (8) has recently completed a map showing the geographical distribution of the disease in the state of Massachusetts, and a table showing the number of elms known to be affected in each town, during 1935 and 1936. During these two years the Shade Tree Disease Laboratory at Massachusetts State College received approximately 5,000 specimens for diagnosis of disease. Of this number approximately seventy-five per cent were collected from elm trees.

Ths total number of trsss found to bs infected by Cephalosporium sp. was 337[2].

FIELD OBSERVATIONS

Limited field observations by the writer have been supplsmented by extensive field notss of trained scouts and othsr collaborators. The disease itself is characterizsd by a gradual wilting and drying of ths leaves, espscially on ths tsrminal twigs. In the summsr the appearance of yellowsd foliags or "flags" in the crown is usually ths first observed sign of ths presence of ths dissase. Later the leaves die, turn brown and roll in from the margins toward the upper surface of the leaf. Ths death of the twigs is evidenced by a drooping, drying out, and tendency of the terminal leavss to persist for some time. Usually these symptoms appear first in a small ssction of ths crown and gradually enlarge as the dissase spreads from the smaller branches to the largsr. Goss and Frink (7) describe the yellowing as beginning at the base of the leaf and gradually spreading up along the midrib, then out along ths veins to ths margins; this being followed by the browning of the tissues and ths death of the leavss from the margins inward.

2. The tabulatsd results of the geographical distribution of the Cephalosporium fungus in Massachusetts ars includsd in a tabls and map as a part of this paper (sss fig. 1 and Table I)

Figure 1.

Map showing geographical distribution of Cephalosporium sp. Towns in which the disease was found are shown in red.

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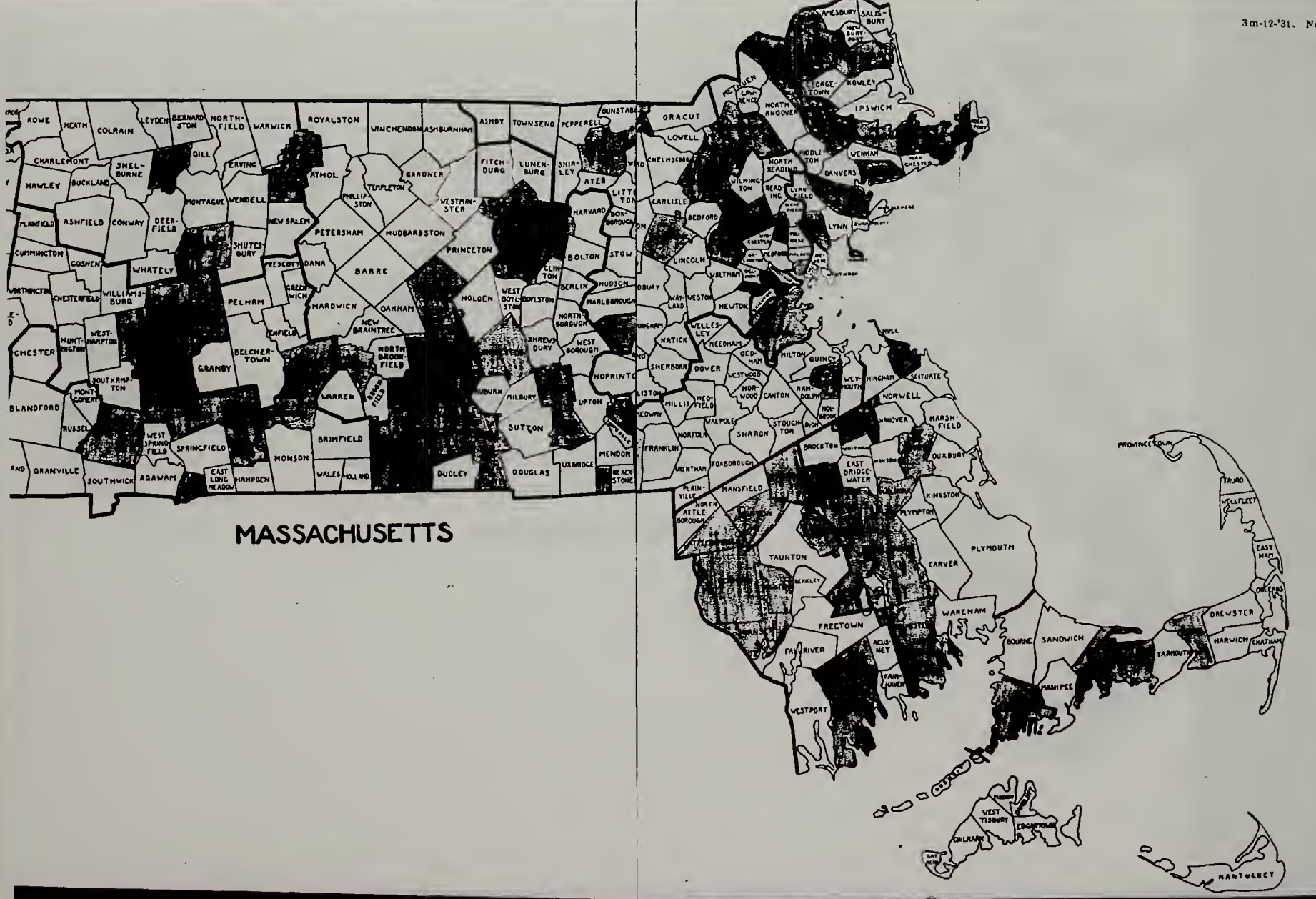
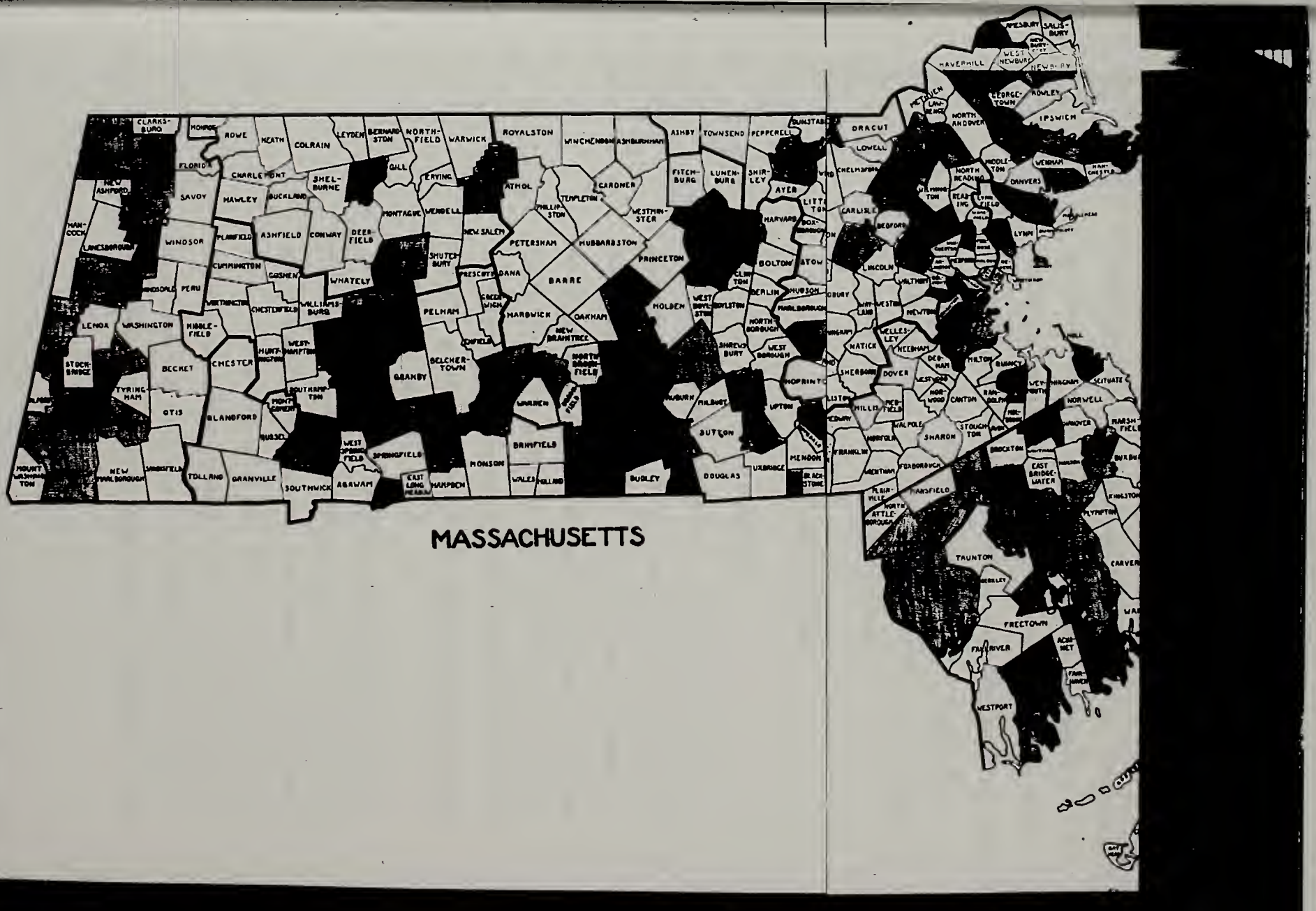




MASSACHUSETTS

The internal symptoms usually precede the external; the former consist of a streaking of the wood in affected twigs, particularly in the spring wood (fig. 2). The streaks are brown and often much more pronounced than those caused by Ceratostomella ulmi (Schwartz) Buisman. In trees where the disease is well established the discoloration often forms a solid ring, but where the disease is less serious the streaking may be confined to one side of the twig or may form a discontinuous ring in the spring wood.

LABORATORY STUDIES

The Fungus

The morphology of the genus Cephalosporium is described by Buchanan (5) as being characterized by its well-developed hyaline mycelium and its slender, unbranched conidiophores which abstrict non-septate spores from the tip. These spores are pushed to one side by the development of later spores. The spores produced in this manner from the tip of a conidiophore are stuck together by mucus and thus remain as a head.

The mycelium is in all cases hyaline, at least when young, septate and much branched. The sterile hyphae are of indeterminate length. The diameter of the hyphae varies from 5 to 25 microns. The cell contents, at first homogeneous, became somewhat vacuolate and later hold a large number of oil drops. The hyphae cross and recross repeatedly; they penetrate the medium to a depth of half an

Figure 2.

Diseased Elm twigs showing
characteristic discoloration
in the spring wood.

inch in agar. The organism grows well only in the presence of an abundance of oxygen.

The conidiophores are developed abundantly on all hyphae that lie at the surface of the medium and upon the aerial hyphae when formed. A few develop even below the surface of the medium. They are slender, hyaline and vary in length from a micron or two to twenty or thirty on some aerial hyphae and ten to fifty microns on a moist surface or in a moist atmosphere. They are usually non-septate.

The spores are formed by the abstriction of the tip of the sporophore. Each is enveloped in mucus, the amount depending upon the moisture of the atmosphere in which it develops. In a dry atmosphere only sufficient moisture is found to cause the spores to stick together in a head. In a moist atmosphere the globule of mucus swells until it completely envelops the spores, and careful observation will show the spores floating free in the liquid. This liquid sometimes amounts to three to four times the mass of the spores. The heads vary in size from ten to thirty-five microns and contain from two to numerous spores. The spores usually possess granules and are ovoid to cylindric with rounded ends. When the sporophores are short, the spore masses are found upon the surface of the hyphae. Sometimes, after a sporophore produces a head of spores, because of some undetermined stimulus, it resumes growth and produces a new head. This phenomenon may occur several times and results in masses of spores at intervals along the sporophores.

The spores devsloped on the moist surface of the medium are usually larger than those of the aerial conidiophores. When the epores are formed on the eurface they frequently continue to enlarge after separation from the hyphae and become considerably elongated, even creecent-shaped, and falcate. When grown to several times their original length they become septate, from one to eix or eight septa being formed. These spores then bud at one or more points and develop new conidia of a similar size and shape. In thie manner large masses of allantoid, septate conidia are produced. They remain attached to each other by elender threads. Many of these spore masses in the older portion of the culture are distinctly visible to the naked eye. In an atmosphere sufficiently moist eome of the erect conidiophores are found to be capped by theee long septate spores rather than by the more usual short, non-septate type. Every graduation in shape, eize, and septation may be observed in a single mount from some cultures. The spores borne on aerial conidiophoree and forming heads of the usual type are from four to fifteen microns in length and one half to one third as broad. Those that develop in a moist atmosphere vary from five to fifteen microns and are one fourth to one half as broad as long. When developed on the surface of the medium in the presence of an excess of moisture, they either resemble the preceding or become allantoid or falcate, twenty to thirty by three to five microns.

The fungus obtained by the writer, from the many elm twigs received in the laboratory is characteristic of the Cephalosporium as described by Buchanan. It is extremely variable both in color and in texture. When first isolated from diseased wood the colony may be rich red, pink, reddish brown, orange, grey or white. The color is due mainly to a staining of the agar by the fungus. The colony is cottony and fairly thick while the edge is characterized by feathery irregularities (fig. 3). The transfer colonies tend to vary widely from the original colonies, some become brilliantly colored and very fluffy (fig. 3) while many bleach out and the mycelium becomes more recumbent. The colonies kept many months in culture by transfers, tend to become white in color with some streaks of sickly green or brown, recumbent mycelium and a smooth edge; these in turn may produce brown, reddish brown, or reddish colonies with the mycelium tending to return to its original fluffinsss.

The mycelium gives rise to myriads of erect conidiophores each of which bears a head of spores at its tip. To obtain spores for study a tuft of mycelium was removed from the culture by means of forceps and then washed in a drop of distilled water on a glass slide. The resulting spore suspension was then examined under the microscope. The spores are hyaline and vary in shape from ovoid to fusiform to allantoid (fig. 4). They contain one or two oil globules; the size varies from two to six microns by six to twenty microns with

Figure 1B4

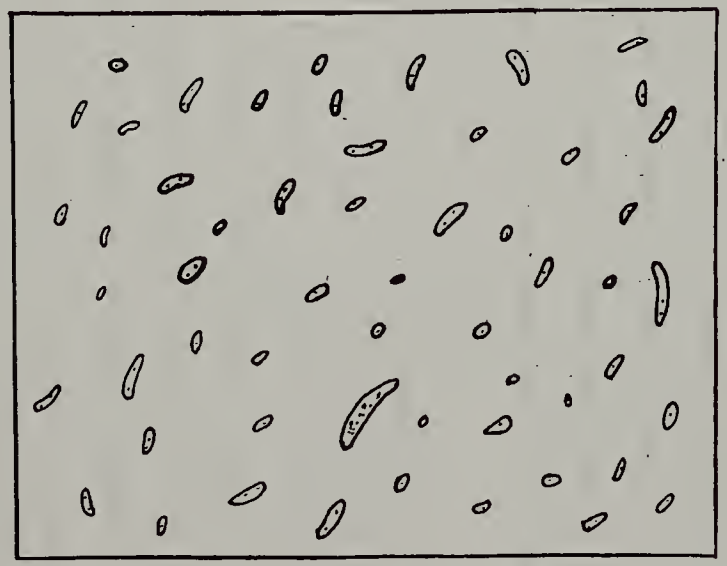
A. Young culture of Cephalosporium sp. made from tissue plantings of discolored elm wood in potato dextrose agar medium (slightly reduced).

B. Typical transfer culture on potato dextrose agar medium, one week old. (slightly reduced)

Figure 4.

Conidia of the causal fungus
of the elm wilt.

(x 1000)



probably a small percentage either larger or smaller. The average size of spores computed from five different cultures is 4.6 by 11.4 microns. (See tables II - VI)

The mycelium is finely branching, hyaline, septate, and it anastomoses frequently. The branching is irregular (fig. 6) with an angle of approximately ninety degrees at the fork. The hyphae may be six microns in diameter but the terminal hyphae are smaller and more thread-like in appearance. Large spore masses similar to those described by Buchanan (5) were found in many cultures, especially those cultures where the aerial mycelium was not abundant.

The ease with which the organism may be cultured is illustrated by an incident which occurred during the summer routine in the laboratory. After cultures had been made, the specimens, in envelopes, were placed in a wooden file until the cultures should be ready for diagnosis. When the specimens were removed for examination with their respective cultures it was found that one drawer was swollen and the specimen envelopes were decidedly moist. In three of these envelopes were twigs the cultures of which showed growth of Cephalosporium sp. These twigs were covered with tufts of mycelium growing out through the lenticels. Cultures were made from the hyphae taken from each specimen. These cultures all yielded growth of Cephalosporium sp. and one twig yielded a pure culture.

The Disease

Small pieces of infected twigs showing pronounced discoloration were prepared for sectioning. The wood was first boiled in water to remove all air, part of the wood was then imbedded in celloidin according to Wetmore's (16) adaptation of Jeffrey's method. The rest of the wood was sectioned dirsctly without imbedding. All of the twigs were sectioned with the sliding microtome and it was found that, for the purposes of the present studies, celloidin embedding, and boiling for direct sectioning were equally satisfactory. Some of the sections were stained with Haidenheims Haematoxylon and Safranin. However, it was found that unstained sections not only yielded much better results, but also were simpler to prepare. Whether or not imbedding of diseased twigs in celloidin and detailed staining of sections might be more valuable in studies extending over a relatively long period of years, of course, could not be speculated from the limited observations reported here.

Fungous hyphae were found to be present in the vessels and tracheids of the discolored areas. The vessels were clogged with gummy, brown tyloses throughout the infected area. The result of infection of the host by the fungus is, thsrefore, a typical vascular mycosis. (fig. 5).

Figure 5.

Longitudinal section of infected elm wood showing fungus hyphae and tyloses in the vessels.

(x 800)

With the possibility in mind that the fungus might be sprsad by spores within the vssssls, measuremsnts of the width of vesssls and trachsids in the elm wood wers made, in the early part of the summer of 1935. It was found that in the spring wood the averags width of the vessels, between the two inner walls, was 32.5 microns, while the corresponding measurement of the tracheids was 20.6 microns. Thsse figures were computed from the measurements made of 35 tracheids and 35 vessels. (Table VII) The width of thess elements were found to be relatively conetant, so that it wae not conaidered necessary to make a greater number of measurements. Since the average sizs of the spores was found to be 4.6 by 11.4 microns the figures arrived at, together with observations of the fungus spread in experimental tress also reported in this paper, seemed to justify the supposition that the fungus might easily be spread inside the tree by means of spores.

In a paper entitled "The distribution of spores of wilt-inducing fungi, throughout the vascular system of the elm by the sap stream", which he presented at the American Association for the Advancement of Science meetings in December 1936, Banfield (3) arrived at a similar conclusion rsgarding ths spreading of spores within elms.

EXPERIMENTAL METHODS

The Fungus

Spore suspensions were made from agar cultures by pouring sterile water into the petri dish in which the culture was growing and transferring the spore suspension thus formed to sterile test tubes by means of sterile pipettes. From this suspension hanging drops were prepared in sterile water and in dextrose solution, the suspension was also smeared on thin discs of potato dextrose agar in Van Tiegham cells under sterile conditions. All of these preparations were kept in sterile petri dishes with distilled water in the bottom to prevent drying out of the media. Those spores which were in the dextrose solution and those in the sterile water germinated in seventy-two hours (fig. 6). The growth was very slow and the writer could find no evidence of branching hyphae from these spores; they ceased growth after a few days. On potato dextrose agar the spores germinated in twenty-four hours and normal branching growth followed (fig. 6).

Spores which were incubated on the agar in darkness were found to germinate readily. Those which were incubated on agar where it was light during the daytime, but were exposed to direct sunlight for only a short time during the day were incubated for two weeks, but did not germinate.

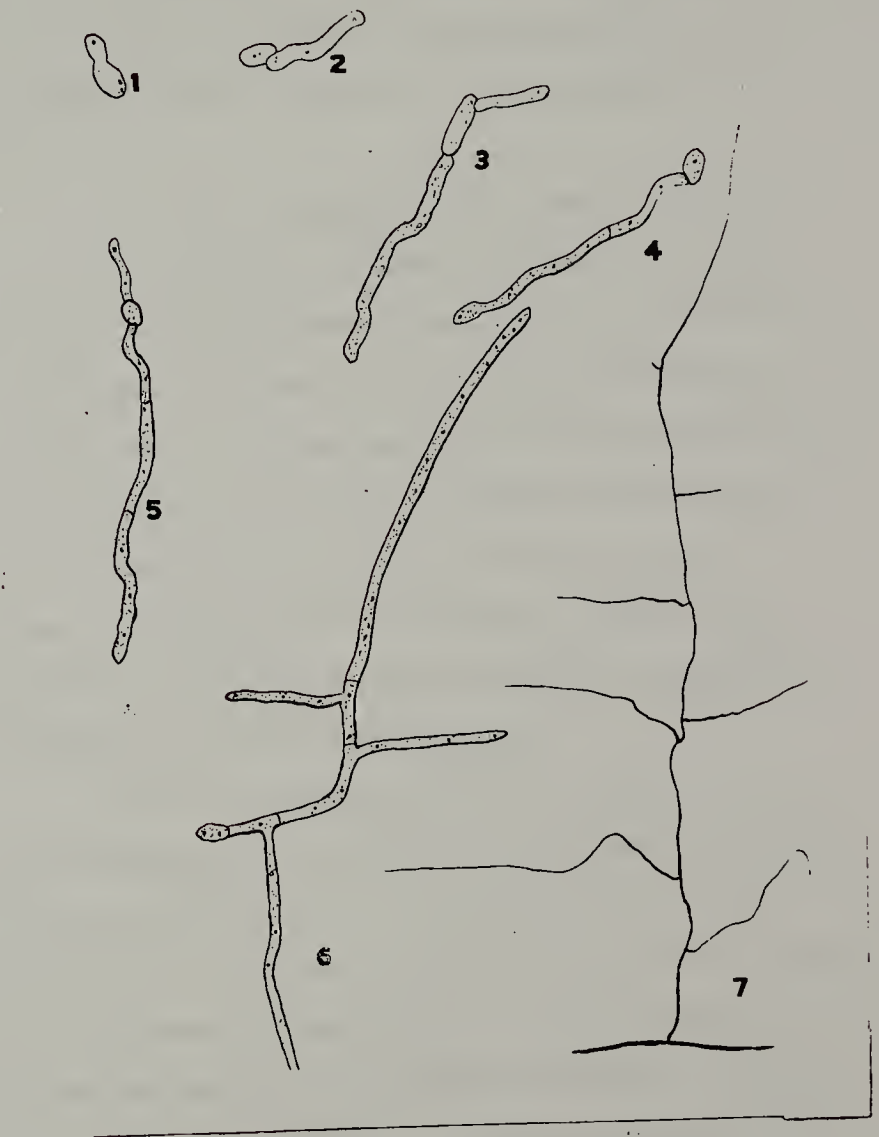
Figure 6 .

Spore Germination

1-5. Germinating spores (x 1000).

6. Germinating spore showing first branching (x 1000).

7. Typical terminal branch of mycelium in Cephalosporium sp.


1
2
3
4
5
6
7

Spores which had already germinated were exposed to the above conditions and at the same time; at the end of the two weeks period the mycelium had grown profusely and extended to the edge of the Van Tiegham ring. The spores which had been incubated in the light for two weeks were placed in ths dark and incubated there for ten days, but no germination was obtained.

In an effort to determine the thermal death-point of the spores, five cubic centimeter portions of the spore suspension previously described, were placed in sterile test tubes and heated in a water bath at varying temperatures for ten minutes each. The tubes were then cooled rapidly and the contents poured into petri dishes containing potato dextrose agar, and incubated for five days to allow the spores to germinate. It was found that the number of viable spores had decreased markedly at seventy-five degrees Centigrade and had disappeared entirely at eighty dsgrees.

Test tubss containing five cubic centimeters each of spore suspension wers subjected to a temperature of minus twsnty degrees Centigrade for varying periods of time; the suspension was then warmed rapidly, poured onto potato dextrose agar and incubated. Spores subjscted to this temperature for as long as twenty-eight days showed no decrease in viability.

The same strain of the fungus which was used in the inoculations, to be described presently, was grown on various culturs media under identical physical conditions.

On potato dextrose agar, the organism grew one inch in seven days; the growth was cottony and white. On the same medium, with lactic acid added, the growth was slightly slower, being 3/4 inch in the same period. On nutrient agar the growth was one inch, the center of the colony was very fluffy, whereas the edge was thin and recumbent. The growth on prune agar was one inch and recumbent. On malt agar the growth was 7/8 inch while the mycelium was coarsely fluffy and quite sparse. On corn meal agar the fungus grew 7/8 inch, the colony was definitely zoned, very sparse, and with many spore masses visible to the naked eye. On oatmeal agar the growth was very similar to that on prune agar and the measurement was also one inch.

The same strain was grown on potato dextrose agar at varying temperatures in order to determine the effect of temperature on the physical properties of the colony. At twenty-five to thirty degrees centigrade, cultures which had been isolated from wood as grey cottony colonies grew one centimeter in twenty-four hours and were white and recumbent, with the edge of the colony very even.

At twenty degrees the mycelium grew fourteen millimeters in one week; it was slightly more fluffy than when grown at higher temperatures. At ten to fifteen degrees the growth was five millimeters in one week, and the mycelium was white and recumbent.

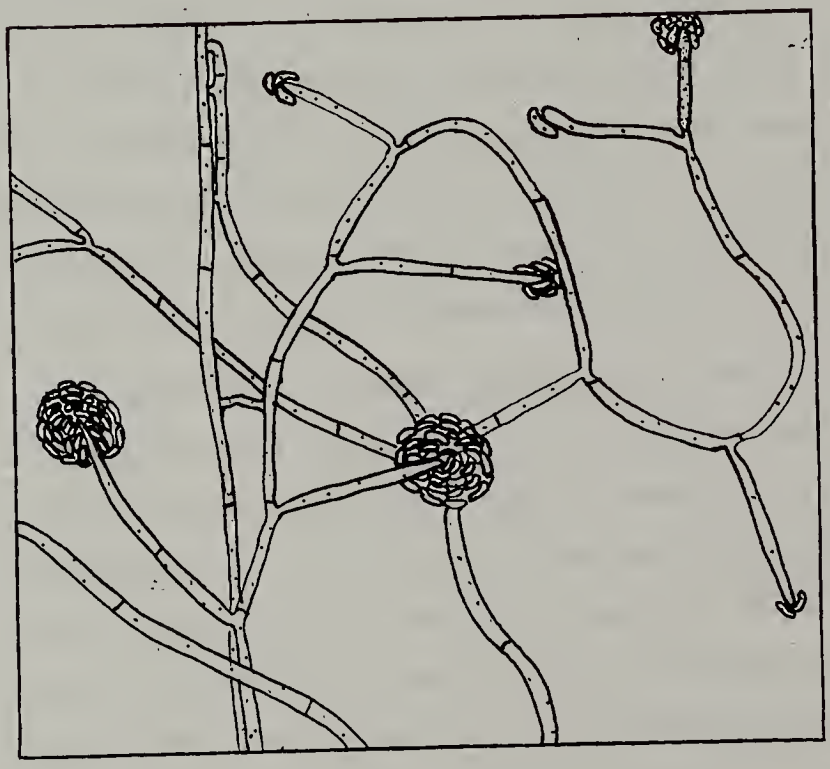
The formation of spore heads (fig. 7) in the Van Tiegham cells, where the spores were germinated on potato dextrose agar, occurred within two to three days after the germination of the spores. All three types of conidiophores described by Buchanan[3] (5) were formed. There were some aerial spore heads, some sub-surface spore heads, and there were innumerable free-floating spores on the surface of the agar. The spore masses, of which he speaks, were not observed in these preparations, but were found in great quantity on the surface of many of the petri dish cultures, some of these masses were as large as two millimeters in diameter.

The spore heads are borne on short conidiophores which abstrict the spores from the tip. The spores cling together to form a head. As few as one or two spores to a head were observed in some cases, while most heads contained many spores. Some heads were highly refractive because of the large quantity of mucus. A very few conidiophores were observed which had more than one head, forming a chain of several spore heads.

3. This description is given in the preceding section under laboratory studies, pp. 6-8

Figure 7

Mycelium growing in Van Tieghem cell showing spore heads and anastomosing hyphae. (x1000)



The Disease

Methods of Infection

For use in inoculations a culture of the fungus as nearly typical as could be found, was selected. This culture had been obtained from twigs which had been sent into the laboratory from the town of Northbridge. The tree from which the twigs were taken was situated on the East Douglas Road near the town line; the twigs had been collected by Achod Ahmadgian, July 23, 1935. The tree was described as being approximately twenty-five feet high and at that time not seriously affected by the disease.

Single spore colonies from this culture were obtained by the streak method; i.e., a spore suspension was made in sterile water and streaked on the surface of a petri dish by means of a sterile platinum loop; any isolated colonies which resulted were then transfered to fresh petri dishes. Transfers of these cultures were made and incubated in petri dishes on potato dextrose agar and also in flasks containing elm twigs and sterile water. Twig media of each of the species and varieties of elm used in the experiments were prepared. The inoculations from twig cultures in the following experiments were made from the medium which corresponded to the tree being inoculated.

Young elm trees, four to six feet in height, of each of the following species and varieties were obtained from a

nursery and grown in earthen crocks in the greenhouse: Ulmus americana, U. americana (var. ascendens), U. campestris L., U. glabra L. var. fastigiata Rehd., U. parvifolia Jacq., and U. pumila L.

1. One tree of each of these species was inoculated, July 15, 1936 in the following manner (fig. 8): A section of bark on the main trunk was washed with alcohol and allowed to dry, then slit vertically with a sterile scalpel for a distance of about three centimeters. The bark was then gently loosened from the wood along either side of the cut and a small piece of bark from a twig culture and on which the fungus was growing vigorously was inserted beneath the loosened bark. The wound was bound with sterile wet cotton which was held in place by a celluloid cylinder. The cotton was kept wet with sterilized water for about two weeks and then allowed to dry out, after which the cylinder and cotton were removed from the tree. Checks were prepared by following the above procedure but omitting the introduction of the fungus-laden bark into the wound and substituting sterilized bark.

A duplicate set of trees was inoculated, on the same day, in the manner described above, but with a slice of agar, on which fungus was growing, substituted for the fungus-laden bark.

Figure 8

Trees in greenhouse with inoculation cylinders, (left) on leaf, and (right) on trunk.

2. One tree of each of the six species was inoculated, July 31, in the following manner (fig. 10). Alcohol was brushed over a leaf and allowed to evaporate; the surface of the leaf was scratched with a sterile scalpel so as to injure the epidermal tissue, and in some places pierce the leaf. A suspension of spores and mycelium, prepared from an agar culture in sterile water, was poured over the leaf, with care taken that some of the liquid remained on the leaf. The leaf was then placed between pieces of wet cotton and rolled in a celluloid cylinder. The cylinder was covered with newspaper, to avoid a possible burning of the leaf tissue, and supported by string in such a manner that the leaf might remain as nearly as possible in a natural position. The cotton was kept moist with sterile water for two weeks, then dried out and removed.

The procedure was repeated on another leaf with the exception that the leaf was not injured. Another leaf was injured and treated in the same manner with the exception that a small block of agar on which the fungus was growing was substituted for the spore and mycelium suspension. A fourth leaf was inoculated with the agar block but left uninjured. Careful checks employing sterile water and agar respectively were employed.

3. Five seedlings of U. americana L. which had been collected from an area of natural seeding, were inoculated July 15, 1936 as follows: two trees were inoculated using

the insertion method previously described, with bark from an elm twig culture as the inoculum for one and a small block of agar on which the fungus was growing as the inoculum for the second. Three trees were inoculated by placing the inoculum in contact with wet cotton supported by a celluloid cylinder. For one tree bark from a twig culture was used as the inoculum, while in the case of the other two the inoculum was a block of fungus-laden agar. These inoculations were kept moist for two weeks, then dried out and the cotton and cylinders removed.

4. Another series of inoculations was carried out with fifteen different isolations of the fungus. These cultures were used directly from isolations without any attempt to obtain single spore cultures. All of the cultures were less than a month old. Inoculations were made on seedlings of U. americana L. by the agar insertion method. Roots also were inoculated by placing a small block of fungus-laden agar on the root tissue from which the epidermis had been scraped. Following this operation the soil which had been previously removed from the roots was carefully replaced. These inoculations were made August 10, 1936.

5. Because of the early falling of the leaves in the first attempt to infect elms through the leaves, a second experiment was initiated, February 24, 1937, with a slightly different technique. Four young potted seedlings of U. americana L. were placed under a bell jar in the greenhouse;

the leavee of two of the treee were injured while the leavee of the other two were left uninjured. A epore suepension wae poured over the surfacee of the leaves of all four trees with care taken that the suepensi on touched both the upper and under eidee. Two similar seedlings were placed under a similar bell jar; the leaves of one of these was injured, eterile water wae poured over the leaves of both trees and they were used as checke. The bell jare were covered with newspaper to protect the treee from the eun. Theee papers were removed after two daye time and the bell jare were removed after ten daye.

Hoet Reaction to Infection

In the first of the above experiments, July 15, 1936, it wae noted that the firet treee to show definite wilting were the two American elms, with perhape, a slightly quicker reaction in the tree inoculated from the twig culture. A month after inoculation the leader of the former had died back for a distance of about forty centimeters, the other terminal twigs ehowed eome evidence of drying out, and the leavee had fallen from the drying twigs. At the same time the latter tree showed similar eymptoms with the exception that the leader had died back for only about thirty centimeters. All other trees in the experiment showed no eymptoms at this time.

The dying-back of the two American elms continued throughout the winter and on the 14th of January, 1937, collections were made for cultural and morphological studies. It was found that on the first tree the fungus, as evidenced by streaking, had progressed upwards thirteen centimeters from the point of inoculation and fourteen centimeters downward. The following spring new shoots sprouted from the base of the tree, but the trunk had died back to within twenty centimeters of the ground (fig. 9), approximately twenty centimeters from the point of inoculation. The second tree was entirely dead at the time of collection, however, no streaking was evident and the author was unable to isolate any fungus from the wood, even at the point of inoculation. It was assumed that the death of the tree was due to maladjustment to the conditions imposed by growing the tree under unnatural conditions, and this tree was discounted in considering the results of the experiment.[4]

The two trees of U. americana L. (Var. ascendens) evidenced some drying near the tip of the terminal shoots. In the tree inoculated from the twig culture the discoloration extended three centimeters upward and two centimeters downward. The fungus was reisolated from the streak.

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4. Some of the trees received from the nursery did not grow well in the crocks, but the majority of the trees which were not used in inoculations showed no ill effects. For this reason, it was not considered an unfair test to experiment with trees growing in crocks.

Figure 9.

American elms which died back after inoculation with the causal fungus, Cephalosporium sp., was reisolated from the pruned leaders. Photographs show new sprouts from below the infected wood.

In the tree which had been inoculated from an agar culture the streaking extended two centimeters upward and two centimeters downward; the fungus was reisolated from this discoloration also.

In English elms (U. campestris L.) neither tree, showed any external symptoms of the disease. Only one tree, that which had been inoculated with the twig culture, showed discoloration; in this the streak extended four centimeters upward and two centimeters downward. The fungus was isolated from the tree showing discoloration but not from the one which showed no discoloration.

The specimens of Moline elm (U. glabra L. var. fastigiata Rehd.) obtained for the experiment were in very poor condition, when received. Of the two inoculated, one died shortly after the inoculation; the other, which was inoculated from the twig culture, was dead at the final inspection. It showed no extension of the discoloration, but the fungus was reisolated from the point of inoculation after a period of six months within the host. These results were not considered as a fair test of the susceptibility of this host and have not been considered in the analysis of results from artificially induced infections.

Neither of the two trees of U. parvifolia Jacq. exhibited any external symptoms of the disease. There was no internal discoloration in the tree inoculated from the agar culture and cultures made from the point of inoculation did not yield fungus. The tree which had been inoculated from

the twig culture showed discoloration three centimeters upward and one centimeter downward; the fungus was reisolated.

The Siberian elms (U. pumila L.) also showed no external symptoms. The tree inoculated from the twig culture showed discoloration for four centimeters upward and seven centimeters downward; the tree infected from the agar culture showed streaking two centimetere upward and three centimetere downward. The fungus was reisolated from both trees.

The check showed no symptoms of the disease.

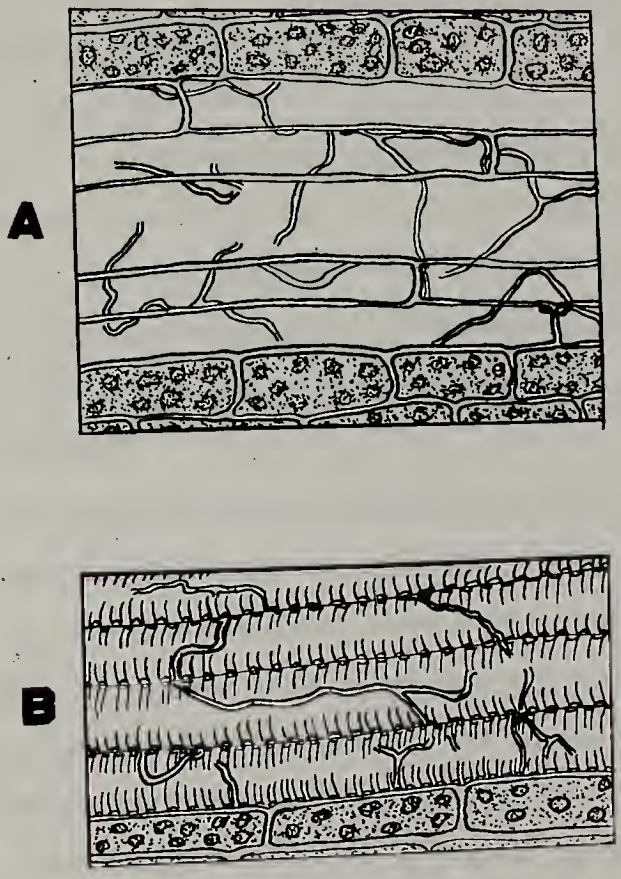
In the second experiment, July 31, 1936, the inoculated leaves fell from the trees when the inoculation cylinders were removed. The leaves were cultured, but with very little success, for it was impoesible to free the cultures from contamination which over-ran the plates. The leaves were examined carefully for any discoloration which might indicate the growth of the fungus in the leaf tissue. All of the leaves which had been injured showed a darkened area around the injuries, while those which had received no injury showed no eymptoms of parasitic activity. The discolored portione of the leaves were sectioned longitudinally with the freezing microtome. These unstained sections were carefully examined under the microecope, and it was found that in all caees the fungus had entered the leaf and was growing in the woody tiseues of the veins (fig. 10). Sections similarly prepared from leaves which had not been injured showed

Figure 10

Longitudinal sections of leaf veins showing hyphae of the causal fungus in the xylem. (x 800)

A. Ulmus americana

B. Ulmus pumila


A
B

no localized discolorations and no fungus was found in the leaf tissue. Cross sections were also prepared from the leaf with similar results. It was found more difficult, however, to discover the fungous mycelium in a cross section of the leaf than in the longitudinal section. The mycelium did not progress as far as the twigs and therefore did not infect the rest of the tree.

In the third experiment, July 15, 1936, the inoculated seedlings of American elm all died within five months. The fungus was isolated from those which had been inoculated under the bark, but the writer was unable to obtain the fungus from the trees which had been inoculated without wounding.

In the fourth experiment, August 10, 1936, cultures were made from twigs which had been collected December 15, 1936 from inoculated trees showing symptoms of the disease. Seven trees showed dying back and from six of these seven the fungus was reisolated. The organism was reisolated from six more trees on April 8, 1937, giving a total of twelve successful infections out of fifteen inoculations.

In the fifth experiment, February 24, 1937, those leaves which had been injured and inoculated soon developed necrotic areas around the wounds, which enlarged to a width of about 1.5 millimeters from the edge of the wound; the leaves then gradually became mottled and yellow. One leaf turned brown, the edges curled upward, and it finally dropped

from the tree, April 12, 1937, less than two months after inoculation (fig. 11). Another leaf, on the same date, had turned very brown from the tip to about half way up the midrib toward the petiole; this dry part was decidedly curled and brittle, while the other half of the leaf still showed some green color. The leaf was still clinging to the tree.

The leaves which had been uninjured showed no evidence of the entry of the fungus, but presented the same appearance as the two checks.

DISCUSSION

The widespread distribution of a wilt disease of elm associated with the genus Cephalosporium is becoming increasingly evident to those workers interested in the diseases of our principal shade trees. Its common occurrence in Massachusetts indicates the importance of a thorough understanding of the problem which is raised by its very general distribution.

Reisolations of the causal fungus accomplished six months after inoculation from the following elms: American (Ulmus americana), English (U. campestris), Chinese (U. parvifolia), Siberian (U. pumila), and the ascendens variety of the American (U. americana (var. ascendens)) together with the observed entrance of the fungus into their leaves seems to be sufficient evidence that these trees are all susceptible to the disease. The fact that the fungus, as evidenced by discoloration in the wood, does not develop as rapidly in the

Figure 11

Infected leaf from elm seedling showing necrotic areas around inoculation wounds.

(x 3)

exotic epecies of elm would suggeet, however, that these varietiee are not affected ae seriouely by the dieeaee ae is the common American elm (U. americana) and could be considered partially resietant.

The resulte obtained in the inoculatiohs of the Siberian elms (U. pumila) are directly opposed to the findinge of Goss and Frink (7) in Nebraska. These workers report having inoculated 18 trees of U. pumila by hypodermic injections and by etem and root incieions with uniformly negative results. This writer, however, found no difficulty in infecting two treee of this eame epecies at the firet attempt.

Gose and Frink (7) discount the importance of leavee as a court of infection and euggeet that the dieeaee is probably spread by ineecte which feed on the young twige. They found that the treee which had not been injured in the leaves or twigs were infected as readily ae thoee which had been wounded. Creager, (6) on the other hand, in investigatione carried out at the Arnold Arboretum of Harvard Univereity, finde that the leaves are the moet common infection court, and that the fungue enters only through wounds. He reports having traced the fungus from the leaf through the vaecular etrande of the veins, midrib, and petiole into the etem.

The present experiments confirm the findings of Creager (6) as opposed to thoee of Goes and Frink (7). The fungus hae been found to enter the leavee and twigs when theee organs are wounded but not when they have been left intact.

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5. The idea of a vascular parasite obtaining entrance through the leaves is not new; it has been known for some time that rust fungi associated with the malformation of the woody parts of pines enter through the leavee; eg. in galls and White Pine Blister Rust.

It has also been demonstrated that the mycelium will grow in the veins and midrib of the leaf. The progress of the fungus in the leaf follows very closely that described by Creager (6).

The wide variability of the cultures of Cephalosporium sp. which have been observed by the writer, together with the fact that both the present experiments and those of Creager (6) have been carried out with cultures isolated in New England as opposed to the experiments by Goss and Frink (7) with cultures isolated in Nebraska, gives rise to the suggestion that the cultures used by Goss and Frink (7) may be a different strain or even a different species than those used by Creager (6) or the writer.

The experiments conducted by the writer concerning the pathogenicity of the various strains cannot be considered conclusive. That the strains tested do grow in the host is evident, but to determine the relative pathogenicity of the various strains, or to separate, definitely, one strain from another, would require much more time and careful study. The problem is one of greatest interest, but is, necessarily, outside the scope of the present study.

Most studies of plant diseases are undertaken with the hope that some method of control may be reached, and the present study is no exception. The fact that the fungus will not enter a tree in any way but through a wound is suggestive. However, nothing can as yet be advised as a means of definite

control, but it would seem reasonable that well tended trees, which are protected from insects and kept in a generally healthy condition, would be lsss open to infection than trees which are neglected. Success in cutting out the infection after the tree has once become infected would be hindered by the spread of the fungus within ths vessels by means of spores.

SUMMARY---

1. A wilt disease of elm associated with a fungus belonging to the genus Cephalosporium Corda is found to have become widespread in Massachusetts.

2. Because of the inestimable value of our elms as our principal shade trees it has been considered important that a more thorough knowledge of the disease be gained.

3. Speciee of the genus Cephalosporium Corda which have received attention in scientific literature are, with a very few exceptions, saprophytic.

4. The association of a species of thie genus with an elm disease was first reported in 1931 and the information available concerning the disease is very meagre.

5. The distribution of the diseaee in Massachusetts is presented by means of a map and table.

6. The external symptoms of the disease are typical of die-back diseases.

7. The internal symptoms consist of brown streaks in the spring wood.

8. The morphology of the genus Cephalosporium as described by Buchanan is compared with that of the Cephalosporium used by the writer.

9. Aerial mycelium will grow on infected twige when the twigs are stored in a moist atmoephere.

10. The disease itself is a typical vascular mycosis.

11. Measurements of the spores and of vessels and tracheids, and microscopic studies of the fungus in the tissue, support the hypothesis that the fungus may be spread by means of spores within the tree.

12. Spores are found to germinate most readily on potato dextrose agar, in darkness, and in a moist atmosphere.

13. The thermal death point has been placed between 75^{0} and 80^{0} centigrade.

14. Freezing was not found to exert any influence on the viability of the spores.

15. The effect of various culture media on the growth of the colonies was studied.

16. Sporogenesis is typical of the genus Cephalosporium.

17. Inoculations were made from single colonies in stems and leaves of elm trees. The following species, and varieties of elm were used: U. americana L., U. americana L. (var. ascendens), U. campestris L., U. glabra L. var. fastigiata Rehd., U. parvifolia Jacq., and U. pumila L.

18. Infection was successful in wounded leaves in all cases; and all the trees, with the exception of U. glabra var. fastigiata, were infected in the twig inoculations.

19. The writer has been successful in infecting U. pumila though Goss and Frink (7) have reported complete failure in this respect.

20. The findings of the writer concerning the leaves as an infection court agree with those of Creager (6) as opposed to those of Goss and Frink (7).

21. It is suggested that there may be several strains, varieties, or species of the genus Cephalosporium associated with the die-back of elms in the United States.

22. No definite control measures can be recommended.

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APPENDIX I.

TABLE I

Town	No. of Trees	Town	No. of Trees
Abington	1	Longmeadow	1
Adams	1	Ludlow	5
Amherst	2	Marion	2
Andover	2	Mattapoiset	3
Attleboro	1	Merrimac	2
Barnstable	1	Middleborough	1
Beverly	1	Milford	1
Billerica	1	Millville	17
Boston	3	Monterey	3
Boxford	1	Nahant	7
Braintree	2	Newbury	2
Bridgewater	3	New Bedford	4
Brookline	3	North Adams	2
Burlington	1	Northbridge	2
Cambridge	2	Northampton	14
Charlton	5	Norton	1
Cheshire	3	Orange	1
Chicopee	3	Oxford	1
Cohasset	1	Palmer	1
Concord	2	Paxton	3
Dalton	1	Peabody	1
Dartmouth	3	Pembroke	1
Dedham	1	Pittsfield	1
Dennis	1	Raynham	1
Dighton	1	Richmond	2
East Brookfield	1	Rehoboth	3
Easthampton	5	Rochester	1
Easton	4	Rockland	1
Egremont	9	Rutland	1
Essex	1	Salem	1
Falmouth	3	Saugus	1
Gloucester	2	Seekonk	1
Grafton	1	Sheffield	18
Great Barrington	9	Somerset	4
Greenfield	4	Southborough	17
Groton	2	Southbridge	2
Groveland	1	South Hadley	3
Hadley	4	Spencer	1
Halifax	5	Sterling	1
Hamilton	1	Stoneham	2
Hatfield	3	Sturbridge	3
Haverhill	3	Sunderland	1
Holyoke	43	Swansea	1
Lakeville	1	Tewksbury	3
Lancaster	2	Topsfield	1
Lee	12	Tyngsborough	3
Leicester	3	Ware	2
Leominster	1	Watertown	1
Leverett	1	Webster	1
Lexington	1	West Bridgewater	3

Town	Number of Trees
West Brookfield	3
Westfield	2
West Newbury	4
Williamsburg	1
Williamstown	1
Woburn	2
Worcester	1
	337

TABLE II

Spore Measurements

Culture #1 — Average 4.7 x 12.2 microns

IN MICRONS

width	length	width	length	width	length
4	12	6	18	4	8
6	14	6	18	4	12
8	16	4	8	4	8
4	10	4	12	4	12
4	8	4	8	4	14
6	16	4	12	6	16
6	12	6	12	6	20
4	6	4	20	6	12
4	12	4	8	6	20
4	12	6	12	4	12
4	10	6	12	4	6
4	12	4	8	4	8
4	8	8	16	6	12
4	10	4	8	4	16
4	8	4	12	6	20
4	8	4	12	6	12
4	8	4	8	4	16
4	12	6	12	4	12
4	10	4	10	4	16
4	8	6	12	4	10

TABLE III

Spore Measurements

Culture #2 Average 4.2x11 microns

IN MICRONS					
width	length	width	length	width	length
4	12	2	8	4	8
4	16	4	8	6	12
4	10	4	8	4	8
4	10	6	16	4	16
4	12	6	14	4	16
4	8	4	8	4	12
6	12	4	12	4	8
4	8	4	12	4	10
4	16	4	12	4	6
4	16	4	10	4	10
4	12	2	8	4	16
4	8	2	6	4	12
4	10	4	10	4	12
4	6	4	8	4	10
4	10	4	12	4	16
4	16	4	12	2	8
4	12	4	16	4	8
4	12	4	10	4	8
4	10	4	10	6	14
4	16	4	12	4	12

TABLE IV

Spore Measurements

Culture #3 Average 4.6 x 11.5 microns

IN MICRONS					
width	length	width	length	width	length
6	16	4	12	4	8
4	8	4	12	4	10
6	12	4	12	4	10
4	10	4	10	4	8
6	16	6	12	6	10
4	10	4	12	6	16
4	12	4	10	6	8
6	16	4	12	4	8
6	12	4	10	4	10
6	12	4	10	4	10
4	12	4	8	2	10
4	8	4	12	4	10
4	12	6	12	6	12
4	12	4	12	6	16
4	12	6	14	4	8
4	12	6	16	4	16
6	16	6	14	4	8
4	10	6	14	4	8
4	8	6	16	4	12
4	12	6	12	4	8

TABLE V

Spore Measurements

Culture #4 Average 4.7 x 11.2 microns

IN MICRONS					
width	length	width	length	width	length
4	12	6	12	4	10
4	8	4	8	6	12
4	12	6	12	4	12
6	18	6	16	4	12
6	16	6	12	4	10
4	8	6	20	6	14
6	16	6	20	4	12
4	10	6	12	6	12
6	16	4	8	6	12
4	16	4	10	6	12
4	10	4	8	4	12
4	8	4	12	4	12
4	8	6	12	4	8
4	10	4	8	4	8
4	8	4	8	6	8
4	12	6	12	4	10
2	14	4	12	4	8
6	12	6	14	4	8
6	12	4	12	4	6
4	12	4	8	4	16

TABLE VI

Spore Measurements

Culture #5 Average 4.7 x 11.3 microns

IN MICRONS					
width	length	width	length	width	length
6	12	4	8	4	10
4	14	4	8	4	12
6	12	4	10	6	10
4	8	4	12	4	12
6	8	4	10	4	8
4	16	6	10	4	8
4	8	4	10	6	12
4	8	4	8	4	12
6	10	4	12	4	12
4	16	4	8	6	16
6	16	6	12	6	12
6	12	4	14	4	10
6	12	6	12	6	16
4	10	6	16	4	10
4	10	6	12	4	8
6	12	4	10	4	10
4	12	6	12	4	10
6	16	4	8	4	12
4	12	4	10	6	12
4	12	4	12	6	8

TABLE VII

Width of Inside of Cells

Tracheids average 20.6 microns		Vessels average 32.5 microns	
width in microns		width in microns	
24	20	44	24
20	16	32	28
24	20	40	32
20	24	36	32
16	20	28	28
24	16	40	40
24	24	40	32
20	16	32	32
20	20	32	32
20	24	36	24
24	16	32	36
24	20	32	40
20	20	28	32
20	20	32	28
24	20	32	28
20	16	32	32
20	20	28	28
16		32	

APPENDIX II

The Need for Further Work

The foregoing paper presents studies of a problem on a disease of elm which has been shown to be one of sufficient importance and scientific concern to deserve careful study. The studies which have been discussed have served to outline more clearly the work which should follow.

The experiments reported by the writer have extended over a relatively short period of time. Therefore, further studies of the disease would be most advantageous.

The experiments have been carried on entirely in the greenhouse, but supplementary studies out-of-doors would aid in a more complete understanding of the disease.

Experiments with different species of elms have been very interesting although somewhat limited. Additional studies might be carried out with a much larger number of trees. The fact that in some cases only one of the two trees inoculated developed the disease would suggest that the idea of finding resistant individuals is not without foundation.

The variability of the fungus in culture is of very great interest. A study of the factors involved in producing changes in the cultural appearance of the fungus concerned should prove to be important.

The question of controlling the disease is also a matter which should be considered in future studies.

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Date May 27, 1937.